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Research paper

How the composition and manufacturing parameters affect insulin release from polymeric nanoparticles

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ABSTRACT

Polymeric nanoparticles (PLA-PCL-Eudragit® RS-ethylcellulose-PVA) containing insulin were prepared by a double emulsion (W/O/W) solvent evaporation technique. The quantification of insulin in the particles was performed by HPLC analysis; the particles were characterized by different techniques, namely DSC, SEM and infrared analysis. The parameters of the preparation process were optimized. The encapsulation efficacy could be increased up to 99%, using PCL 60000/Eudragit® RS. The nature of the polymers also affected the size and zeta potential of the nanoparticles and insulin release kinetics. The use of ethylcellulose in the formulation and ultrasound during nanoparticle preparation allowed obtaining particles smaller than 300 nm.

1. Introduction

The use of polymeric nanoparticles for therapeutic or diagnostic purposes is one of the most active areas of research. It is motivated by the need to help a drug in crossing biological, chemical and biochemical barriers of the body, with a view to arriving intact and in adequate quantity at the target. Nanoparticles are submicron solids, the role of which is often to encapsulate and assure drug transport to the site of action. Ideally, these structures are biocompatible. Biocompatibility is the property of a material to perform its function without causing local or systemic adverse effects [1].

Nanoparticles can allow, in the case of therapeutic molecules, to protect the active ingredient in the gastrointestinal tract, to overcome problems of low solubility, to increase the therapeutic efficacy, or to reduce their immunogenicity [2,3]. In the case of proteins such as insulin, polymeric nanoparticles might allow its oral administration by protecting insulin along the digestive tube, vis-à-vis degradation due to acidity.

The most widely used polymers for the encapsulation of proteins are poly (lactic acid), PLA; poly (glycolic acid), PGA; and copolymers such as poly (lactic co glycolic acid),PLGA, and poly(ε-caprolactone) PCL. However, it has been reported that PCL leads to denaturation of proteins [4]. The mixture PCL/polyacrylic acid can lead to preservation of the biological activity of insulin and its prolonged release in vivo [5].

Our study is devoted to the preparation and micro- and nanoparticles made of bioresorbable and metabolizable polymers such as poly(lactic acid) (PLA), poly (ε-caprolactone) (PCL), ethylcellulose and blends of PCL/Eudragit® RS, PCL/PLA and PCL/ethylcellulose containing insulin. Polymeric microcapsules were obtained using a double emulsion and solvent evaporation method, which is often used for encapsulating proteins and peptides [6].

2. Material and methods

2.1. Reagents

The following materials were used without further purification.

- Polymers: Poly (ε-caprolactone) (PCL) ($\overline{M}_W$ = 10,000, 60,000; 80,000), Ethylcellulose (EC) ($\overline{M}_W$ = 150,000) and Polyvinyl

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alcohol (PVA) ($\overline{M}_W$ = 30,000, 88% hydrolyzed) were obtained from Sigma Aldrich.

Eudragit® RS (ERS) ($\overline{M}_W$ = 150,000) was a generous gift from Evonik.

Poly (lactic acid) L9000 (PLA) ($\overline{M}_W$ = 220,000, 92% L-lactide and 8% D-lactide) was purchased from Sigma Aldrich.

- Human aqueous Insulins (100 IU/ml: 1UI is 0,035 mg) were directly bought from retail pharmacies.

Quick Insudal®: human Insulin biogenetic fast action (Saidal Group).

Insulin Aspart Novorapid®: fast acting insulin (insulin analog).

Actrapid® (Novo Nordisk): fast acting human insulin produced by recombinant biotechnology.

- Solvent: Dichloromethane (CH_2Cl_2) (BiochemChemopharma)

2.2. Preparation of polymeric nanoparticles

The preparation of polymeric nanoparticles was performed by the solvent evaporation method as described by Damgé et al. [5]. 1 ml of insulin (Insudal, Actrapid or AspartNovorapid) was dispersed in 10 ml of CH_2Cl_2 containing 250 mg of polymer (PCL, PLA) alone or a mixture of polymers (PCL/PLA, PCL/ERS or PCL/EC), under ultrasonic agitation for 30 s. Thereafter, the emulsion was dispersed in 40 ml of an aqueous solution of 0.1% PVA constituting the continuous phase, under ultrasonic agitation for one minute. CH_2Cl_2 was evaporated under reduced pressure using a rotary evaporator for 10 min at 40° C. The obtained nanoparticles were separated by centrifugation at 42,000 g for 20 min at 20° C and then lyophilized and stored at 4° C.

2.3. Particle characterization

- Analysis of the particles size distribution and zeta potential was achieved by means of a Zetasiser Malvern Instrument (FTA/MAL Zetasizer 1041949).

The encapsulated insulin level was determined by liquid chromatography using a Shimadzu HPLC apparatus brand and monitored by a kinetic study in vitro at pH = 7.4 and Tp = 37° C.

- Infrared analysis (FTIR) was performed using a Shimadzu spectrophotometer brand 8101 M on KBr pellets containing 1–3% of insulin nanoparticles as freeze-dried powder in comparison with bulk polymers as references.
- The morphology and rough estimate of the size of the capsules were characterized using a scanning electron microscope (SEM) type EIF, with different magnifications.

2.4. Release kinetics in vitro

For each formulation, insulin release was measured in three media: at pH 1.2, 6.8 and 7.4.

For the preparation of PBS buffer pH = 7.40.6 g of KH_2PO_4, 6.4 g Na_2HPO_4 and 5.87 g NaCl were dissolved in 1 L of distilled water (and adjusted to pH 7.4with orthophosphoric acid if necessary). The release medium simulating the gastric contents were prepared as follows: 2 g NaCl were dissolved in 80 ml of 1 M HCl and water added up to 1 L. The pH was adjusted to 1.2 with 1 M HCl. The "intestinal medium" was prepared as follows:6.8 g of K_2HPO_3 were dissolved in250 ml of water mixed with 77 ml of NaOH 0.2 M. The, water was added up to 1 L. The pH was adjusted to 6.8 with 0.2 M NaOH.

50 mg nanoparticles (as freeze-dried powder) were dispersed in 20 ml of PBS buffer at pH = 7.4 in a thermostatic bath at a temperature of 37° C under magnetic stirring at 50 rpm.1 ml of each solution was sampled at different times: 5 min, 30 min, 1 h, 2 h, 4 h, 6 h, 8 h and 24 h. Afterwards, the volume of the solution was readjusted to 20 ml by addition of 1 ml of PBS. After centrifugation, the samples were assayed by HPLC (Shimadzu; UV–Vis detector: Model SPD 20A, L20134200950 Series LP). The mobile phase was composedof25% acetonitrile-TFAand75%water-TFA (TFA: trifluoro acetic acid; 1 ml TFA in1 liter of each eluent). The flow rate was 1 ml/min.

3. Results and discussion

3.1. Estimation of particle size and zeta potential by DLS

The physicochemical properties of the nanoparticles (size, size distribution, surface morphology and surface zeta potential) should be determined systematically before considering an application of the formulation, regardless of the mode of delivery. Nanoparticles were first characterized from a physicochemical point of view in terms of size and zeta potential, carried on by dynamic light scattering (DLS) method. Table 1 summarizes the average size values and zeta potentials of the various formulations investigated. The sizes of the insulin-loaded nanoparticles were between 245 nm and 817 nm. Nanoparticles prepared from polyesters (PCL, PLA) exhibited a negative zeta potential due to the presence of deprotonated carboxylic functions at the surface of the nanoparticles. PCL nanoparticles with Actrapid® insulin are negatively charged; the zeta potential was −5.94 mV, which is correlated with the work of Socha et al. [7].

This potential increased upon adsorption of positively charged polymers to the surface of the nanoparticles, such as Eudragit® RS (polycationicmethacrylic type); (due to the positively charged quaternary ammonium groups) [2,3]. The PCL/ERS (50/50 weight to weight) blend formulation obtained with Insudal® insulin possessed a zeta potential of between 27.1 mV and 31.7 mV, whereas that obtained with Actrapid® and AspartNovorapid® had a zeta potential greater than 40 mV.

The ethylcellulose formulation with insulin was negatively charged (Insudal®: −4.13; Actrapid®: −18.84; AspartNovorapid®: −3.93 mV), as already reported in literature [4]. The use of ethylcellulose is known to increase the encapsulation rate of water-soluble drugs. Thus, we have chosen to use it in combination with to control drug release based on PCL biodegradation. The corresponding formulation, PCL_{10000}/EC, was negatively charged, the value of the zeta potential was about −6.75 mV with Insudal®, and −7.03 mV with AspartNovorapid®, but an even lower value was obtained with Actrapid® (−16,20 mV).

The formulations which were prepared with PLA only or with a PCL_{10000}/PLA mixture, had much lower zeta potential than PCL/EC-Insulin and EC-Insulin nanoparticles; this is due to the association of the two polymers (PCL, PLA), which showed a better stability (−25mv).

Using a double emulsion and solvent evaporation method, the physicochemical properties of the particles obtained are directly influenced by certain parameters. For example, an increase in polymer concentration leads to an increase of the size and the polydispersity of the particles.

Other parameters such as the nature and the molecular weight of the polymer used and the additives (excipients) alter the efficiency of the encapsulation and release kinetics of the drug.